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ENDOCRINE INFLUENCES ON SEMINAL VESICLES IN THE ESTUARINE GOBIID FISH, *GILLICHTHYS MIRABILIS*

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Seminal vesicles, which occur as paired glandular structures attached to the posterior region of the common spermatic duct in male teleost fishes, were noted by Rathke (1824) in the goby, *Gobius niger*. Since then, seminal vesicles have been observed and briefly described in several other gobiid fishes (Hyrtl, 1850; Eggert, 1931; Weisel, 1949; Tavolga, 1955; Egami, 1960) as well as in many other fishes (Hyrtl, 1850; Disselhorst, 1904; Gudger, 1908; Ihering, 1937; Gilbert, 1943; Sundararaj, 1958; Nawar, 1959; Hoffman, 1963; Sircar, 1966, Lehri, 1967; Rastogi, 1969; Nayyar and Sundararaj, 1970a). The seminal vesicles of *Gobius paganellus* (Vivien, 1938), *Heteropneustes fossilis* (Sundararaj, 1958), *Clarias lazera* (Nawar, 1959), *Opsanus tau* (Hoffman, 1963), and *Mystus tengara* (Rastogi, 1969) exhibit seasonal development and regression closely following changes in the testes. On the other hand, Weisel (1949) has reported that in *Gillichthys mirabilis*, an estuarine gobiid fish, the seminal vesicles do not show cyclicity; the amount of secretion and the appearance of the secretory epithelium do not change appreciably in the various seasons.

The literature on the endocrine regulation of teleost sex accessories was reviewed by Pickford and Atz (1957), Dodd (1955, 1960), Hoar (1957, 1969) and Bern and Nandi (1964). However, data on endocrine influences on the secondary sex accessories such as the seminal vesicles are meager. The seminal vesicles of teleosts are not homologous with their mammalian counterparts inasmuch as they do not arise from the Wolffian duct. There is no unanimity of opinion with regard to the response of the seminal vesicles to androgen. Weisel (1949) indicated that the seminal vesicles of *Gillichthys mirabilis* do not respond to testosterone. Sundararaj and Goswami (1965a, 1965b), Sundararaj and Nayyar (1967, 1969a, 1969b, 1969c) and Nayyar and Sundararaj (1969, 1970b) have presented a detailed picture of endocrine control of the seminal vesicles in the catfish, *Heteropneustes fossilis*.

The seminal vesicles of *Gillichthys mirabilis* do not show cyclical changes, whereas the testes have a distinct annual cycle (de Vlaming, 1972). This indicates that the secretory activity in the seminal vesicles may be regulated by a direct pituitary principle in addition to possible testicular influences. Ths investigation on the estuarine gobiid fish, *Gillichthys mirabilis*, was undertaken (1) to study the effects of castration or hypophysectomy on the secretory seminal vesicles and, (2) to determine the response of the seminal vesicles of the hypophysectomized speci-

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mens to administration of testosterone propionate and ovine prolactin, singly and in combination.

Materials and Methods

Specimens of *G. mirabilis* were collected in January from the Alviso salt ponds at the southern end of San Franciso Bay in California; this habitat was previously described and the reproductive cycle of this population of *G. mirabilis* educidated (de Vlaming, 1971a, 1972).

Due to the large gape of *Gillichthys,* hypophysectomy can be performed by entering through the mouth. The hypophysis of this fish is situated just posterior to the first gill cleft in the cranial floor. After removal of a small section of the buccal epithelium, the gland is readily visible as a white oval body. Fish were anesthetized with 1:4000 tricaine methane sulfonate (MS 222) dissolved in sea water. Animals were then placed belly upward in a groove made in a large cork. The lower jaw was held open with a hook attached to a ringstand. After cutting away a section of the buccal epithelium, a small hole was made in the parasphenoid bone just anterior to the pituitary with a (No. 5) dental drill. Two slits were then cut posteriorly from this hole and the bone folded back. The pituitary was removed by vacuum. The bone was then folded back into place and covered with buccal epithelium. Fish were returned to sea water following surgery. For further details on hypophysectomy of *Gillichthys* see de Vlaming (1971b).

Fish to be castrated were anesthetized with MS 222. A short incision was made to the left of the mid-ventral plane in the lower abdomen. Due to the loose skin of *Gillichthys* this incision could be moved so that each of the testes could be observed. The testes could be easily removed through the incision by grasping them firmly with forceps. The sperm duct leading from each testis was cut approximately 3 mm anterior to the junction with the seminal vesicle. The incision was carefully sutured with surgical silk and the area cleaned with 70% ethanol. This procedure has been thoroughly discussed by Sundararaj and Goswami (1965b).

Hormone treatments were begun seven days after hypophysectomy. Each fish received a total of seven daily injections and were sacrificed the day after the last injection. Castrate fish were sacrificed 14 days after castration. Testosterone propionate (Mann Research Laboratories) was injected in 1% solution of Tween and ovine prolactin (NIH-P-S8) in a 0.6% saline solution. The volume injected was kept constant at 0.1 ml. All experimental fish weighed between 35 and 50 g. The experimental groups and hormone dosages are presented in Table I.

Experimental fish were maintained in 56 liter fiber glass tanks. These tanks were provided with recirculating filtered sea water. Animals were fed every other day on chopped beef liver. Further details on maintenance of *Gillichthys* can be obtained in de Vlaming (1971b, 1971c). Water temperature was regulated at 18° ± 1° C since this temperature promotes reproductive activity (de Vlaming, in preparation) and is within the preferred temperature range of this species (de Vlaming, 1971a). The lighting schedule was 13 hr of light and 11 hr of dark.

Upon termination of the experiments, fish were killed with a saturated solution of chlorotone and weighed. The seminal vesicles were dissected out, weighed and

TABLE I

Effect of testosterone propionate (TP) and prolactin (LtH) on the seminal vesicles of hypophysectomized Gillichthys mirabilis

Group No.	n	Treatment for 7 days, daily dosage		Seminal vesicle Wt. (mg)/Body wt. (g) ($\bar{x} \pm$ S.E.)*	Area of 8 largest vesicular lumina (mm^2) ($\bar{x} \pm$ S.E.)**
		TP (μg)	LtH (μg)		
Sham operated					
I	5	0	0	2.84 ± 0.28	47.53 ± 4.16
Hypophysectomized					
1	6	0	0	0.38 ± 0.08	16.15 ± 4.00
2	6	25	0	0.11 ± 0.02	1.67 ± 0.67
3	6	150	0	1.49 ± 0.35	35.06 ± 4.87
4	6	0	200	0.96 ± 0.21	44.21 ± 7.02
5	6	25	200	4.00 ± 0.96	60.12 ± 7.59

* P values (Mann-Whitney U test): I and 1: $P < 0.001$; I and 2: $P < 0.001$; I and 3: $P < 0.01$; I and 4: $P < 0.001$; 1 and 2: $P < 0.05$; 1 and 3: $P < 0.01$; 1 and 4: $P < 0.05$; 1 and 5: $P < 0.001$; 2 and 3: $P < 0.01$; 2 and 4: $P < 0.01$; 2 and 5: $P < 0.001$; 3 and 5: $P < 0.05$; 4 and 5: $P < 0.01$.

** P values: I and 1: $P < 0.001$; I and 2: $P < 0.001$; I and 3: $P < 0.05$; 1 and 2: $P < 0.001$; 1 and 3: $P < 0.01$; 1 and 4: $P < 0.001$; 1 and 5: $P < 0.001$; 2 and 3: $P < 0.001$; 2 and 4: $P < 0.001$; 2 and 5: $P < 0.001$; 3 and 5: $P < 0.05$; 4 and 5: $P < 0.05$.

weights were standardized by dividing them by the body weight of the fish. The seminal vesicles fixed in alcoholic Bouin's fluid and embedded in paraffin for histological examination. Sections were cut 8–9 μ and stained with Harris' hematoxylin and eosin. Camera-lucida drawings were made of the eight largest lobules of the seminal vesicles of each fish. The area of the lumina of these lobules was determined using a planimeter. Mean area was then determined for each experimental group (see Table I).

Statistical comparisons between experimental groups were made by using the Mann-Whitney U test (Siegel, 1956). This nonparametric test is suitable for small sample sizes and can be used to determine whether two independent groups have been drawn from the same population.

RESULTS

Weisel (1949) has described the seminal vesicles of *G. mirabilis*. In the sham-operated fish, the seminal vesicles were enlarged and distended with secretion. The secretory epithelium was hypertrophied (Table I).

Fourteen days after castration, the seminal vesicles of four of the five castrate fish were regressed so completely that they could not be easily dissected out. The weight (mg seminal vesicles/g body weight) of the seminal vesicles of the remaining castrate fish was 0.43. No colloid remained in the lobules of the seminal vesicles of this fish and the secretory epithelium was degenerated.

Hypophysectomy also caused a regression of the seminal vesicles. The weight of the seminal vesicles and area of the vesicular lumina in the 14-day hypophy-

sectomized fish were significantly less ($P < 0.001$) than in the sham-operated controls (Table I). Very little colloid remained in the lobules of the hypophysectomized fish and the secretory epithelium was beginning to degenerate.

The effects of the various hormone treatments are summarized in Table I. A low dose (25 μg/day) of testosterone propionate (TP) did not inhibit seminal vesicle regression in the hypophysectomized fish (Table I). Rather, it accentuated regression. On the other hand, a higher dose (150 μg/day) of TP did prevent regression of the seminal vesicles in the hypophysectomized specimens. This treatment maintained the secretory epithelium and colloid was present in the lobules. The seminal vesicle weight and area of the lumina of the lobules in the TP-treated (150 μg/day) group were significantly greater ($P < 0.01$) than in the hypophysectomized control fish. Seminal vesicle activity in this group, however, was slightly less than in the sham-operated controls (Table I).

Treatment with mammalian prolactin also prevented regression of the seminal vesicles in the hypophysectomized specimens. The vesicular epithelium was well-maintained and colloid filled the lumina. Nonetheless, the seminal vesicle weights in the prolactin-treated group were significantly less ($P < 0.001$) than in the sham-operated group, but the areas of the lobule lumina were not significantly different in these two groups. Compared to the hypophysectomized controls, seminal vesicle weights ($P < 0.05$) and luminal areas ($P < 0.001$) were significantly greater in the prolactin-treated group. The seminal vesicles of the prolactin-treated fish and those of fish receiving a high dose (150 μg/day) of TP appeared to be in a similar condition.

Treatment with a combination of prolactin and a low dose of TP (25 μg/day) was effective in maintaining seminal vesicle activity at a level comparable to that in the sham-operated controls (Table I). The seminal vesicle weights and luminal area were significantly greater in this group than in those receiving either TP or prolactin alone (see Table I for P values).

Discussion

In the gobiid fish, *G. mirabilis*, castration or hypophysectomy causes regression of the seminal vesicles. Regression of similar structures following hypophysectomy was also observed in several other fishes (Vivien, 1938, 1941; Tavolga, 1955; Sundararaj and Goswami, 1965b; Sundararaj and Nayyar, 1969a, 1969b). Our data thus suggest that the seminal vesicles in *G. mirabilis* are androgen dependent; hypophysectomy may cause regression by reducing or inhibiting androgen production by the testes. In contrast to our results with *G. mirabilis*, however, Sundararaj and Goswami (1965b), Sundararaj and Nayyar (1969b, 1969c) and Nayyar and Sundararaj (1969) noted that castration during the prespawning period stimulated hypersecretory activity in the seminal vesicles of the catfish, *Heteropneustes fossilis*. These workers found that the high titers of gonadotropin following castration stimulated the interrenal to produce androgens which in turn acted on the seminal vesicles to induce hypersecretion (Nayyar and Sundararaj, 1969; Sundararaj and Nayyar, 1969b, 1969c).

In *G. mirabilis* treatment with testosterone propionate (TP) or ovine prolactin prevents regression of the seminal vesicles in the hypophysectomized fish. But

neither of them alone was effective in maintaining the seminal vesicles at the level of activity noticed in the sham-operated controls. Sage and de Vlaming (unpublished data) have also noted that prolactin inhibits regression of the seminal vesicles in the hypophysectomized *G. mirabilis*. On the other hand, Weisel (1949) reported that the seminal vesicles of *G. mirabilis* do not respond to testosterone; his experiments, however, were with intact fish injected with testosterone only three times at 7-day intervals or suspended in the aquarium water. More experimental data are needed to determine whether or not both hormones promote growth and secretion in the completely regressed seminal vesicles. Sundararaj and Goswami (1965b) and Sundararaj and Nayyar (1969a) have reported that in the hypophysectomized catfish, *Heteropneustes fossilis,* prolactin alone does not initiate secretory activity in the seminal vesicles, whereas androgen alone is effective (Sundararaj and Goswami, 1965a; Nayyar and Sundararaj, 1970b).

Only a combination of prolactin and TP was effective in maintaining the seminal vesicles at the level of activity seen in the sham-operated controls. Sundararaj and Goswami (1965b) and Sundararaj and Nayyar (1969a) have shown that prolactin promotes growth and secretion in the seminal vesicles of the catfish, *Heteropneustes fossilis*, only in the androgen-primed fish; thus, their data suggest a synergism between prolactin and testosterone—neither hormone being as effective as a combination of the two. A similar synergism is apparently operative in *G. mirabilis*.

Prolactin in synergism with low levels of androgen (which alone do not stimulate seminal vesicles) is very effective in maintaining the secretory seminal vesicles of the hypophysectomized *G. mirabilis*. In the fish treated wth prolactin alone, the possibility of low titers of androgen being released from the testes or interrenal cannot be ruled out. The above suggestion at least in part may explain the continued activity of the seminal vesicles even at a time when the testes are regressed. Since prolactin is capable of synergizing with very low levels of androgen, seminal vesicles may presumably be activated or at least maintained whenever prolactin is released. In *G. mirabilis* prolactin brings about dispersal of pigment in the xanthophores (Sage, 1970). Further, the fish when placed in fresh water turns yellow—an indication of prolactin release, although there is no evidence that prolactin has an osmoregulatory role in this species (Sage, 1970). Even so, it is not unreasonable to expect the release of prolactin in *G. mirabilis* with decrease in the environmental salinity. *G. mirabilis* is afterall an estuarine fish capable of tolerating wide fluctuations in salinity. If prolactn is at all involved in osmoregulation, its relase brought about by changes in salinity, would also affect the seminal vesicles even though the testes may be regressed, provided androgen is present. Another possibility is that prolactin stimulates androgen production by either the testes or interrenal. Although this suggestion seems unlikely, Ball and Fleming (unpublished data cited in Ball and Ensor, 1969) and Chambolle (1967) have shown that prolactin does stimulate the interrenal in some teleosts.

The lack of any obvious seasonal cycle in the seminal vesicles of *G. mirabilis* indicates that throughout the year the titers of prolactin and/or androgen are sufficient to maintain some activity. Although there is a clear-cut cyclicity in the testes of this fish (de Vlaming, 1972), androgen production by the testes or interrenal may remain at a level sufficient to synergize with prolactin in maintaining

seminal vesicle activity. Further experiments are necessary to examine this possibility.

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Summary

1. The effects of castration or hypophysectomy on the seminal vesicles of the gobiid fish, *Gillichthys mirabilis* were examined. In addition, the influence of ovine prolactin and testosterone propionate treatments was determined in the 7-day hypophysectomized specimens.

2. Both castration and hypophysectomy caused regression of the seminal vesicles,

3. A low dose of testosterone propionate (25 μg/day) did not inhibit regression of the seminal vesicles in the hypophysectomized fish.

4. A high dose of testosterone propionate (150 μg/day) or prolactin treatment prevented regression of the seminal vesicles in hypophysectomized fish; neither treatment, however, maintained the level of activity observed in sham-operated fish.

5. A combination of testosterone propionate and prolactin maintained seminal vesicle activity in the hypophysectomized fish at a level equal to that in sham-operated controls.

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